

## Biomonitoring of workers cleaning up ammunition waste sites

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### Abstract

2,4,6-Trinitrotoluene (TNT) is an important occupational and environmental pollutant. In TNT-exposed humans, notable toxic manifestations have included aplastic anaemia, toxic hepatitis, cataracts, hepatomegaly and liver cancer. Therefore, it is important to develop protection measures and to monitor workers involved in the clean-up of ammunition sites. Haemoglobin (Hb) adducts of TNT, 4-amino-2,6-dinitrotoluene (4ADNT) and 2-amino-4,6-dinitrotoluene (2ADNT), and the urine metabolites of TNT, 4ADNT and 2ADNT were found in 22–50% of the exposed workers, but not in the control group. The exposed workers were wearing protective equipment. The levels of erythrocytes, haemoglobin, creatinine, serum glutamic pyruvic transaminase and lymphocyte levels were significantly lower in the exposed workers than in the non-exposed workers. The levels of blood urea and reticulocytes were significantly higher in the exposed workers than in the non-exposed workers. Headache (26%), mucous membrane irritation (16%), sick leave (18%), lassitude (8%), anxiety (6%), shortness of breath (3%), nausea (5%) and allergic reactions (8%) were reported by the exposed workers. In a further analysis the U-4ADNT levels and the Hb-adduct levels were compared to the blood parameter and the health effects. The blood parameters were not significantly different between the U-4ADNT positive and U-4ADNT-negative group. Headache, mucous membrane irritation, sick leave, lassitude, anxiety, shortness of breath and allergic reactions were statistically not different between the two groups. Also in the workers with Hb-4ADNT adducts no significant negative changes were seen in regards to the changes of the blood parameters or the health effects. According to the results of the present study, it appears that the blood parameter changes and the health effects are more influenced by other factors than by the internal exposure to TNT.

**Keywords:** TNT, ammunition waste, haemoglobin adducts, urine metabolites

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### Introduction

2,4,6-Trinitrotoluene (TNT) is an important environmental and occupational pollutant (Hathaway 1985, Yinon 1990, IRIS 1991, Rosenblatt et al. 1991, ATDSR 1995). In TNT-exposed humans, notable toxic manifestations have included aplastic

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anaemia, toxic hepatitis and cataracts (Hathaway 1985, Rosenblatt et al. 1991, ATSDR 1995). A recent retrospective study on male workers exposed to TNT for more than 1 year from eight Chinese military factories during 1970 to 1995 demonstrated an elevated relative risk for malignant tumours, especially liver cancer (Yan et al. 2002).

*In vitro* studies with rat liver microsomes showed that TNT is rapidly reduced to yield 4-hydroxylamino-2,6-dinitrotoluene (OH-4ADNT), 4-amino-2,6-dinitrotoluene (4ADNT) and 2-amino-4,6-dinitrotoluene (2ADNT) as intermediates that are further metabolized to form 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene (Leung et al. 1995). The *N*-hydroxyarylamine (OH-4ADNT) of 4ADNT was stable enough so that the reduction of TNT to 4ADNT appeared to be a two-step pathway (Yinon 1990, Leung et al. 1995). In contrast, the 2ADNT intermediate, OH-2ADNT, could not be isolated. The oxidation of the methyl group in animals appears to be a minor pathway because such products have been reported only once in the literature (reviewed in Yinon 1990). Secondary products of *N*-hydroxyarylamines are responsible for the genotoxic and cytotoxic effects of these compounds. In mammalian systems the principal metabolites of TNT are 2ADNT and 4ADNT; smaller amounts of other metabolites also have been found (Yinon 1990). Urine metabolites usually indicate only recent exposures up to 48 h post-exposure (van Welie et al. 1992). Covalent haemoglobin (Hb), plasma protein and protein adducts in various tissues were found in rats dosed with radiolabelled TNT (Liu et al. 1992). The mechanism of adduct formation between aromatic amines or nitroarenes with Hb involves the reaction of the metabolite, nitrosoarene, with cysteine residues to form a sulfinamide (Green et al. 1984, Kazanis & McClelland 1992, reviewed in Sabbioni & Jones 2002). Sulfinamides are readily hydrolysed under mild conditions, yielding the parent amine. Hb adducts are dosimeters for the internal dose and possibly for the target dose leading to toxic effects. Hb adducts are an indicator of exposure over the previous 4 months, if the adduct is stable and if the lifetime of the erythrocytes is not affected.

In humans, mainly 2ADNT and 4ADNT were found in urine after TNT exposure (Woollen et al. 1986, Ahlborg et al. 1988, Yinon 1990, Bader et al. 1998, Sabbioni et al. 2005). Hb adducts have been found in workers exposed to TNT (Liu et al. 1995, Sabbioni et al. 1996, Sabbioni et al. 2005, Sabbioni et al. 2006a,b). For the present work we investigated the exposure and health effects of workers cleaning ammunition sites in Germany in order to see if the preventive measure taken were sufficient.

## Materials and methods

### *Workers*

Informed consent was obtained from each worker. The collection of blood and urine, anamnesis, and completion of questionnaires were all performed in the same week. Participants were interviewed with a questionnaire about their general health status, exposure history, smoking habits, previous medical record and present symptoms. The workers for this study were recruited from workers of three projects involved in the clean-up of ammunition waste: Stadtallendorf (43 non-exposed and 41 exposed (12 were follow-ups of non-exposed workers), Espagit-Hallschlag (26 exposed), Clausthal-Zellerfeld (11 non-exposed and 14 exposed (three were follow-ups of the non-exposed workers)), and a control group of workers ( $n=31$ ), who were not

involved in the clean-up of ammunition waste. According to the questionnaire the workers were separated into exposed and non-exposed workers. The workers answered the questions about their workdays at ammunition waste sites in the previous 4 months, the previous 4 weeks and the previous 7 days. Workers with no exposure in the previous 7 days were considered non-exposed. This yields a total 85 (81 males) non-exposed. Of these 85 non-exposed workers, 80 workers were not exposed in the 4 previous weeks, and 76 workers were not exposed in the previous 4 months. A small number of workers ( $n=15$ ) with no exposure were monitored a second time after 3 months and after exposure to ammunition waste. This yields 80 (78 males) exposed workers (15 were follow-ups of the non-exposed workers). The major craftmanship of the workers were (non-exposed, exposed): machinist and engine driver (21, 16), weapon disposal workers (0, 21), foreman (8, 12), craftsman for underground work (18, 2), road and construction worker (11, 6), truck driver (7, 2), construction engineer collecting soil samples (= samplers) (3, 5), sewage workers (4, 0), steel and concrete construction worker (2, 2), artificer (1, 3), and other work duties with less than four workers (11, 12). The workers involved in the Stadallendorf project were mainly from two companies ( $42+35=92\%$ ). The workers at the Espagit-Hallschlag project were all employed by the same company. The other workers originated from an additional 12 companies. The workers from five companies were non-exposed. The mean age of the non-exposed and the exposed workers was  $40.2 \pm 10.2$  and  $41.4 \pm 9.7$  years, respectively. The clinical parameters were determined in 32 non-exposed and 25 exposed male workers. The smoker status was declared by 80 non-exposed and 80 exposed workers. Among the non-exposed (exposed) workers 17 (36) were non-smokers, 51 (32) were smokers and 12 (12) were former smokers. The questions regarding the personal protection measures (Figure 1): breathing protection (masks), gloves, overall, and hand cream were answered by 76–80 exposed workers. Questions about the health status (dizziness, nausea, anxiety or palpitations, headache, mucous membrane irritation of the airways or the eyes, allergic reactions, blood in urine) were answered by 6 non-exposed and 62–63 exposed workers. The medical department of the institution for statutory accident insurance and prevention in the civil engineering sector performed the following examinations: (i) anamnesis; (ii) routine blood tests, (leukocyte count, erythrocyte count, thrombocytes, lymphocytes, neutrophils, haemoglobin, haematocrit,

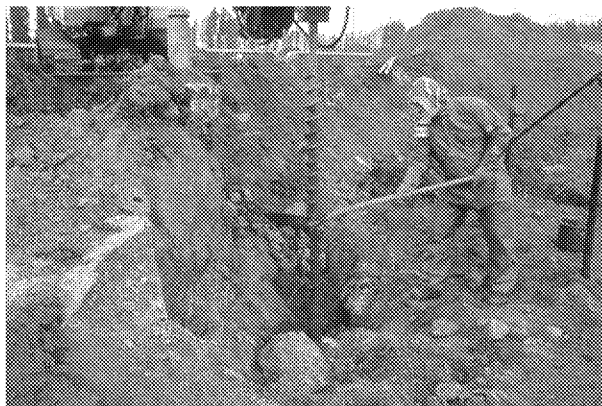


Figure 1. Typical protection measures for workers involved in the clean-up of an ammunition waste site.

mean corpuscular volume (MCV), glucose, creatinine, urea, glucose, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and gamma-glutamyl-transpeptidase (GGT).

#### *Urine metabolites*

Urine metabolites were determined according to the procedure published in Sabbioni et al. (2005). Urine metabolites were determined in 78 exposed and 84 non-exposed workers. Urine metabolites were analysed without acid and/or enzyme pretreatment. The deuterated d<sub>5</sub>-2ADNT and d<sub>5</sub>-4ADNT were used as internal standards. The limit of quantification (LOQ) was 60 pg 4ADNT and/or 2ADNT per urine sample (2 ml).

#### *Hb adducts*

Hb adducts were measured in 80 exposed and 85 non-exposed workers. Hb adducts of TNT were analysed according to the procedures published by Sabbioni et al. (1996, 2005) using 200 mg Hb per analysis. The deuterated d<sub>5</sub>-2ADNT and d<sub>5</sub>-4ADNT were used as internal standards. The LOQ was 30 pg 4ADNT and/or 2ADNT per sample.

#### *Statistical analysis*

Statistical analyses were performed with the SPSS program (version 10.0). The results of the questionnaire and of the medical examination were not known to the scientists performing the analyses of the biomarkers. All results were disclosed at the end of the analyses. For the comparison of one dichotomous dependent variable with a continuous independent variable, the Mann-Whitney test was used. For the comparison of two sets of dichotomous variables, contingency tables were used. The Hb adduct, the urine metabolite, blood sugar, GGT and reticulocyte levels were not normally distributed (one-sample Kolmogorov-Smirnov test,  $p < 0.05$ ). For these parameters comparisons between groups were performed with the Mann-Whitney test and the independent  $t$ -test. All the other blood parameters were normally distributed and comparisons were performed with the independent  $t$ -test.

### **Results**

Workers participating in three clean-up projects in Germany were monitored. Hb adducts and urine metabolites of TNT were determined. The same procedures were performed in a control group of workers not involved in the clean-up of ammunition waste. The exposure status was determined with a questionnaire. The results of the adduct levels and urine levels are listed in Tables I and II. Hb adducts of 4ADNT (Hb-4ADNT) were found in 23% of the exposed workers and in 6% of the non-exposed workers. Hb adducts of 2ADNT were not found in either worker groups. The urine metabolite 4ADNT (U-4ADNT) was found in 50% of the exposed workers and in 23% of the non-exposed workers. The urine metabolite 2ADNT (U-2ADNT) was found in 18% of the exposed workers and in 2% of the non-exposed workers. The range, the median, the 75th, 90th and 95th percentile levels of Hb adducts and urine metabolites are listed in Table I. The Hb-4ADNT, U-4ADNT and U-2ADNT levels were significantly higher in the exposed group than in the non-exposed group

Table I. Haemoglobin (Hb) adduct and urine levels of TNT (2,4,6-trinitrotoluene) metabolites presented as range, median, 75th, 90th, 95th percentile.

	Hb-4ADNT (pg/g Hb)	U-4ADNT (pg/ml)	U-2ADNT (pg/ml)
Exposed	0 <sup>a</sup> -1391, 0, 0, 316, 407	0-1629, 16.1, 92.2, 317, 549	0-278, 0, 0, 46.1, 75.1
Non-exposed	0-315, 0, 0, 0, 174	0-988, 0, 0, 68.5, 144	0-203, 0, 0, 0, 0

<sup>a</sup> <LOQ (limit of quantitation). 4ADNT, 4-amino-2,6-dinitrotoluene; 2ADNT, 2-amino-4,6-dinitrotoluene.

according to the Mann-Whitney test ( $p < 0.05$ ). The adduct and urine metabolite levels in the control group ( $n = 31$ ) were below the LOQ.

The adduct and urine levels were compared with the health effects monitored in exposed and non-exposed workers (Table II). These analyses were performed with contingency tables. Hb-4ADNT and U-4ADNT were significantly more prevalent in the exposed workers. Smokers were more prevalent among the non-exposed workers. Headache, mucous membrane irritation, sick leave, lassitude, anxiety, shortness of breath, nausea and allergic reactions were reported by 26%, 16%, 18%, 8%, 6%, 3%, 5%, and 8%, respectively, of the workers. No health effects were noted in the non-exposed group, except for one case of sick leave.

The same parameters were investigated for the different projects (Table II), companies (Table III) and work task (Table IV) in order to find exposure and health effect clusters. U-4ADNT did not differ among the exposed workers of the three project groups. Headache was significantly more prevalent in the Stadtallendorf project in comparison with the exposed workers of the other projects. In general, health effects were less prevalent for the exposed workers of the Clausthal-Zellerfeld project than for the other exposed workers.

The evaluation of the single worker groups showed that most positive U-4ADNT and Hb-4ADNT levels were found in the road and construction workers. However health effects were observed more in the group of machinists/engine drivers, weapon disposal workers and foremen. Headache was most prevalent in the group of engine drivers. Lassitude, anxiety, shortness of breath, sick leave and mucous membrane irritation were more often present in the engine drivers and the weapon disposal workers in comparison with the other groups. Allergic reactions were more prevalent in the foremen.

The blood parameters were compared between the exposed and non-exposed male workers (Table V). The mean age of the two groups was similar. The levels of erythrocytes, Hb, creatinine, SGPT and lymphocytes were significantly lower in the exposed workers. The levels of urea and reticulocytes were significantly higher in the exposed workers. For some workers the values of blood parameters were above the normal range. However, most abnormal cases were found in the non-exposed workers except for the lymphocyte levels and the mcv values.

A comparison between the exposed workers of two projects showed striking differences. The leukocyte, neutrophil, reticulocyte, urea, glucose, haematocrit and MCV levels were significantly higher in the workers involved in the Espagit-Hallschlag project than in the Clausthal-Zellerfeld project. However, the lymphocyte levels were significantly lower. The abnormal cases of blood parameters were then compared between the projects, the companies and the work duties. Most cases with abnormal GGT and SGPT levels were found in the Stadtallendorf project; in particular, in

Table II. Haemoglobin (Hb) adducts, urine metabolites, protective measures, health effects and smoker status for exposed and non-exposed workers involved in different projects. Statistical significance of the cases was determined with contingency tables: Fisher's exact test, exact significance (two-sided).

	Exposure	Hb-4ADNT Yes/no	U-4ADNT Yes/no	Smoker Yes/no	Gloves Yes/no	Overall Yes/no	Mask Yes/no	Hand cream Yes/no	Headache Yes/no	Mucous membrane Yes/no	Sick leave Yes/no
Stadtallendorf	Non-exp	1/42	15/28 <sup>a2</sup>	25/12	n.d.	n.d.	n.d.	n.d.	0/0	0/0	0/0
	Exp	14/26 <sup>b2,c</sup>	19/19	22/18 <sup>b2</sup>	36/2	40/0	38/2	29/9 <sup>b2</sup>	13/18 <sup>b2</sup>	4/28	4/27
Espagit-Hallschlag	Exp	0/26 <sup>b1</sup>	13/13	7/19	23/3	26/0	25/1	8/17 <sup>b1</sup>	2/17	5/14	5/14
Clausthal-Zellerfeld	Non-exp	4/7	4/7 <sup>a2</sup>	3/8 <sup>a1</sup>	n.d.	n.d.	n.d.	n.d.	0/6	0/6	1/5
	Exp	4/10	7/7	3/11	14/0	14/0	14/0	6/8	1/11	1/11	2/10
Controls <sup>e</sup>	Non-exp	0/31	0/31 <sup>a1</sup>	23/8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total	Non-exp	5/80	19/65	51/28 <sup>d</sup>	n.d.	n.d.	n.d.	n.d.	0/6	0/6	1/5
	Exp	18/62 <sup>d</sup>	39/39 <sup>d</sup>	32/48	73/5	80/0	77/3	43/33	16/46	10/53	11/51

<sup>a1</sup>Prevalent no-cases in comparison with the other non-exposed workers ( $p < 0.05$ ).

<sup>a2</sup>Prevalent yes-cases in comparison with the other non-exposed workers ( $p < 0.05$ ).

<sup>b1</sup>Prevalent no-cases in comparison with the other exposed workers ( $p < 0.05$ ).

<sup>b2</sup>Prevalent yes-cases in comparison with the other exposed workers ( $p < 0.05$ ).

<sup>c</sup>Prevalence of yes-cases between non-exposed and exposed workers of the same project ( $p < 0.05$ ).

<sup>d</sup>Significant difference between non-exposed and exposed workers ( $p < 0.05$ ).

<sup>e</sup>Construction workers not involved in ammunition waste clean up.

4ADNT, 4-amino-2,6-dinitrotoluene; n.d., not determined.

Table III. Haemoglobin (Hb) adducts, urine metabolites, protective measures, health effects and smoker status for exposed workers employed by different companies. All workers of company 6 were involved in the Espagit-Hallschlag project. All workers of companies 1 and 5 were involved in the Stadtallendorf project. Significance of the cases was determined with contingency tables: Fisher's exact test, exact significance (two-sided).

Company	Hb-4ADNT Yes/no	U-4ADNT Yes/no	Smoker Yes/no	Gloves Yes/no	Overall Yes/no	Mask Yes/no	Hand cream Yes/no	Headache Yes/no	Mucous membrane Yes/no	Sick leave Yes/no
1: non-exp	0/9	2/7	6/2	n.d. <sup>c</sup>	n.d.	n.d.	n.d.	0/0	0/0	0/0
1: exp	4/12	8/7	10/6 <sup>a2</sup>	16/0	16/0	16/0	13/3 <sup>a2</sup>	7/6 <sup>a2</sup>	1/13	4/9
5: non-exp	1/10	6/5 <sup>b2</sup>	4/6	n.d.	n.d.	n.d.	n.d.	0/0	0/0	0/0
5: exp	8/5 <sup>a2,b</sup>	9/3	5/8	12/1	13/0	13/0	9/4	4/5	3/6	0/9
6: exp	0/26	13/13	7/19	23/3	26/0	25/1	8/17 <sup>a1</sup>	2/17	5/14	5/14

<sup>a1</sup>Prevalent no-cases in comparison to the exposed workers of all other companies ( $p < 0.05$ ).

<sup>a2</sup>Prevalent yes-cases in comparison to the exposed workers of all other companies ( $p < 0.05$ ).

<sup>b</sup>Prevalence of yes-cases comparing non-exposed and exposed workers of the same company ( $p < 0.05$ ).

4ADNT, 4-amino-2,6-dinitrotoluene; n.d., not determined.

Table IV. Haemoglobin (Hb) adducts, urine metabolites, protective measures, health effects and smoker status for exposed workers with a specific work task. Significance of the cases was determined with contingency tables: Fisher's exact test, exact significance (two-sided).

	Hb-4ADNT Yes/no	U-4ADNT Yes/no	Smoker Yes/no	Gloves Yes/no	Overall Yes/no	Mask Yes/no	Handcream Yes/no	Headache Yes/no	Mucous membrane Yes/no	Lassitude Yes/no	Anxiety Yes/no	Short. breath Yes/no	Nausea Yes/no	Allergicreaction Yes/no	Sick leave Yes/no
Road and construction worker	4/2 <sup>a2</sup>	5/1	3/3	6/0	6/0	6/0	5/1	2/3	1/4	0/5	0/5	0/5	1/4	0/5	1/4
Engine driver	7/14	11/9	10/11	20/1	21/0	20/1	17/4	8/8 <sup>a2</sup>	4/12	2/14	2/14	0/16	1/15	1/15	4/12
Foreman	3/7	6/4	4/6	8/1	10/0	10/0	6/2	3/6	1/8	0/9	0/9	0/9	1/8	2/7	1/8
Sampler	1/2	1/2	0/3	3/0	3/0	3/0	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Weapon disposal	0/19 <sup>a1</sup>	11/8	5/14	19/0	19/0	18/1	5/13	2/13	4/11	2/13	2/13	2/13	0/15	2/13	5/10 <sup>a2</sup>

<sup>a1</sup>Prevalent no-cases in comparison to the cases found in all the other exposed workers ( $p < 0.05$ ).

<sup>a2</sup>Prevalent yes-cases in comparison to the cases found in all the other exposed workers ( $p < 0.05$ ).

4ADNT, 4-amino-2,6-dinitrotoluene.



Table V. Differences of blood parameters in exposed and non-exposed male workers and differences of blood parameters in male workers with and without U-4ADNT (4-amino-2,6-dinitrotoluene) in urine.

	Non-exposed: ( <i>n</i> = 32) Mean (range)	Exposed: ( <i>n</i> = 25) Mean (range)	<i>p</i> -value ( <i>t</i> -test)	Normal ranges <sup>f</sup>	Subjects above normal range (non-exposed/exposed)	Workers with U-4ADNT No/yes (mean)
Age (years)	38.0 (18.2–60.2)	39.7 (23.0–57.5)	>0.05			
Erythrocytes (10 <sup>6</sup> /μl)	5.19 (4.3–6.2)	4.96 (4.4–5.7)	0.033	4.5–5.90	1/0	5.05/5.14
Haemoglobin (g/dl)	16.0 (13.0–18.7)	15.2 (13.6–16.5)	0.004	13–18	1/0	15.6/15.8
Creatinine (mg/dl)	1.13 (0.8–1.5)	1.01 (0.8–1.2)	0.001	0.5–1.2	7/0	1.08/1.07
Urea (mg/dl)	5.96 (2.1–9.0) <sup>a</sup>	6.83 (3.1–10.0) <sup>a</sup>	0.043	10–55		5.98 <sup>a</sup> /6.79 <sup>a</sup>
SGPT (U/I)	20.9 (7–51)	14.4 (6–41)	0.008	0–22	9/2	18.6/17.4
Lymphocytes (%)	37.2 (7–51)	29.7 (16–60) <sup>c</sup>	0.021	25–45	6/4 <sup>c</sup>	34.7/32.9
Reticulocytes (‰)	10.6 (7–19) <sup>b</sup>	19.3 (7–33) <sup>d</sup>	0.002 <sup>g</sup>	4–15	1/14	13.4 <sup>b</sup> /15.6 <sup>b</sup>
SGOT (U/I)	9.2 (6–14)	11.4 (8–18)	>0.05	0–18		10.7/10.9
Leukocytes (/μl)	7662 (4700–17400)	7128 (3600–12200)	>0.05	4000–10000	2/3	7864/6904
Haematocrit (%)	47.8 (38–65)	46.6 (42–50)	>0.05	41–50		46.9/47.6
MCV (μm <sup>3</sup> )	91.7 (83–105)	93.8 (82–105) <sup>c</sup>	>0.05	85–98	4/5	90.7/89.8 <sup>c</sup>
Glucose (mg/dl)	93.5 (53–291)	84.5 (41–137)	>0.05 <sup>g</sup>	70–120		97.4/81.1
GGT (U/I)	60.4 (4–1035)	17.8 (3–73)	>0.05 <sup>g</sup>	6–28	7/3	59.9/18.0
Neutrophils (%)	60.3 (48–91)	66.3 (40–79)	>0.05	45–74		62.4/62.0
Thrombocytes (1000/mm <sup>3</sup> )	224 (132–309)	220.1 (78–310)	>0.05	150–350		226/218

<sup>a</sup>All urea values were below the normal range.

<sup>b</sup>*n* = 28.

<sup>c</sup>*n* = 24.

<sup>d</sup>*n* = 20.

<sup>e</sup>Low levels of lymphocytes were found in two non-exposed and 11 exposed workers.

<sup>f</sup>Hahn (1997).

<sup>g</sup>These parameters were not normally distributed. The results were confirmed with the Mann–Whitney test.

SGPT, serum glutamic pyruvic transaminase; SGOT, serum glutamic oxaloacetic transaminase; MCV, mean corpuscular volume; GGT, gamma-glutamyl-transpeptidase.

company 1, 44% of the workers had abnormally high GGT values. The workers with the highest prevalence are the road and construction workers with 40% abnormal GGT and SGPT levels. The most cases with abnormally high creatinine levels were found in project 1. High leukocyte levels were found in the Espagit-Hallschlag project. The most cases with abnormally high lymphocyte levels were found in the Stadtallendorf and Clausthal-Zellerfeld projects. The workers with the most cases of abnormally low levels of lymphocytes were found in the workers responsible for the weapon disposal in the Espagit-Hallschlag project. Most workers with abnormally high reticulocyte levels were found in the workers of the Espagit-Hallschlag project.

In a further analysis, the U-4ADNT levels and the Hb adduct levels were compared with the blood parameters and the health effects. The blood parameters were not significantly different between the U-4ADNT-positive and U-4ADNT-negative groups (Table VI). Headache, mucous membrane irritation, sick leave, lassitude, anxiety, shortness of breath, and allergic reactions were statistically not different between the two groups (Table VI). Also in the workers with Hb-4ADNT adducts (data not shown) no significant negative changes were seen in regards to the blood parameters or the health effects.

## Discussion

### *Comparison of U-4ADNT and Hb-4ADNT with the data of other studies*

For the present study, the median U-4adnt level of all positive samples ( $>LOQ$ ) was 82.6 pg/ml. In Chinese workers the median level was  $\sim 210$  ng/ml (Sabbioni et al. 2005). In German workers dismantling old munition, the median 4ADNT levels found in raw urine were 230 ng/ml (51 workers) (Letzel et al. 2003) and 710 ng/ml (nine workers) (Bader et al. 1998). The values in the present investigations are 3000 times lower. This might be explained by the fact that the present group of workers were not dismantling munition. In addition the present project was a pilot study where special attention was given to the protective measures (Figure 1). The median level of all workers with Hb adducts was 0.22 ng/g. In Chinese workers the median level was  $\sim 62.1$  ng/g (Sabbioni et al. 2005). In Germany (Ewers et al. 2000), the median Hb-4ADNT level in people living on a formerly used industrial area for the production of TNT was 0.24 ng/g Hb 4ADNT (range  $<LOQ$  -3.4). In matched controls living in non-contaminated areas, the values were similar, 0.08 ng/g Hb ( $<LOQ$  -1.9). Unfortunately, no experimental details were given for the method of determination of Hb adducts. Therefore the results are difficult to compare. However, these Hb-adduct levels are similar to the values of the present work.

### *Comparison of the blood parameters with the data from other studies*

Effects on blood parameters are presented in Tables V–VIII. The haematological effects noticed in the present study were reported previously for TNT-exposed workers (reviewed in ATSDR 1995). Dose-related reductions in Hb (9.9% lower than control) and haematocrit (11.6% lower than controls) and 50% higher reticulocyte counts were reported in exposed workers (Army 1976). In another study, highly exposed TNT workers showed a significant decrease ( $p < 0.05$ ) of Hb concentration, the number of red blood cells and haematocrit (Sabbioni et al. 2005). In the present group of subjects we noticed an increase of the hepatic effect marker SGOT (data not

Table VI. Comparison of workers with or without 4ADNT (4-amino-2,6-dinitrotoluene) in urine. All differences were statistically not significant.

U-4ADNT	Headache Yes	Mucous membrane Yes	Nausea Yes	Sick leave Yes	Allergic reaction Yes	Short breath Yes	Lassitude Yes	Anxiety Yes	GGT High	SGPT High	Creatinine High	Leukocytes High	MCV High	Lymphocytes high	Lymphocytes low	Reticulocytes high
No	20%	13%	0%	20%	7%	3%	6%	6%	22%	19%	14%	11%	14%	39%	26%	21%
Yes	27%	16%	8%	16%	8%	3%	8%	5%	7%	14%	7%	4%	15%	10%	23%	44%

GGT, gamma-glutamyl-transpeptidase; GPT, glutamic pyruvic transaminase; MCV, mean corpuscular volume.

Table VII. Differences of blood parameters in exposed male workers involved in two different projects.

	Espagit-Hallschlag ( <i>n</i> = 14) Mean (range)	Clausthal-Zellerfeld ( <i>n</i> = 10) Mean (range)	<i>p</i> -value ( <i>t</i> -test) <sup>b</sup>
Age	44.4 (25.0–57.5)	33.5 (23–50)	0.007
Leukocytes (/μl)	8243 (4400–12200)	5920 (3600–8800)	0.015
Haematocrit (%)	47.9 (46–50)	45.2 (42–50)	0.005
MCV (μm <sup>3</sup> )	97.6 (91–105)	88.4 (82–96)	<0.001
Glucose (mg/dl)	98.9 (75–137)	65.7 (41–102)	<0.001
Urea (mg/dl)	7.6 (5.6–10)	5.9 (3.1–81)	0.017
Neutrophils (%)	72.2 (56–79)	54.1 (40–65.5)	<0.001
Lymphocytes (%)	21.4 (16–35)	41.2 (23.2–60)	<0.001
Reticulocytes (‰)	23.9 (19–33)	8.3 (7–10) <sup>a</sup>	<0.001

<sup>a</sup>*n* = 6.<sup>b</sup>All parameters were normally distributed.

MCV, mean corpuscular volume.

shown). Morton et al. (1976) reported a statistically significant increase of SGOT in ammunition plant workers when the TNT level in the air increased. In contrast, no significant differences in liver function were noted in a cross-sectional epidemiology study of 626 munitions workers from four plants exposed to TNT when compared to 865 non-exposed controls (Army 1976). We noted a significant decreased SGPT activity in the exposed workers. The same was found in a group of Chinese workers (Sabbioni et al. 2005). In dogs, a decrease of SGPT levels after exposure to TNT was related to hepatomegaly (Levine et al. 1990). For markers of immunological effects, we noted an insignificant decrease of the leukocyte count and a significant decrease of the lymphocyte levels in the exposed workers. In Chinese workers, the number of white blood cells decreased in the highly exposed group in comparison with the other group (Sabbioni et al. 2005). This is in contrast to the increase in lymphocyte numbers seen in nine fatal cases of TNT toxicity in humans (Army 1978).

#### *TNT exposure and risk evaluation*

In Chinese workers (Sabbioni et al. 2005, 2006b), cataracts, hepatomegaly and splenomegaly were more prevalent in the exposed workers than in the factory controls (60% vs. 26%, 22% vs. 12%, 13% vs. 0%). The influence of Hb-adduct levels on the odds ratio (OR) of developing a disease was estimated using logistic regression analyses (Sabbioni et al. 2005). The OR without confounding factors of suffering from cataracts was 6.4-times higher when the level of Hb-4ADNT adducts increased by one log-unit ( $p < 0.05$ ). Similar ORs were observed with hepatomegaly (7.6) and splenomegaly (9.6). From the *z*-values of the logistic regressions the probability ( $p = 1/(1 + e^{-z})$ ) of a negative health effect might be predicted for Hb-adduct levels found in exposed workers (Sabbioni et al. 2006b): (i) cataracts  $z = -2.90 + 1.85 * [\log (\text{Hb-4ADNT (ng/g)})]$ ; (ii) hepatomegaly  $z = -4.94 + 2.022 * [\log (\text{Hb-4ADNT (ng/g)})]$ ; (iii) splenomegaly  $z = -6.03 + 2.26 * [\log (\text{Hb-4ADNT (ng/g)})]$ . Therefore, for the median Hb adducts found in the positive workers this yields a probability of 1.6% for cataract, 0.2% for hepatomegaly and 0.05% for splenomegaly.

Table VIII. Subjects (non-exposed+exposed) with abnormal blood parameters found in the different projects, companies and work specification were compared. Significance of the cases was determined with contingency tables: Fisher's exact test, exact significance (two-sided).

	GGT High/ normal	SGPT High/ normal	Creatinine High/ normal	Leukocytes High/ normal	MCV High/ normal	Lymphocytes High/ normal	Lymphocytes low/ normal	Reticulocytes High/ normal
Stadtallendorf	7/21	8/20	6/22 <sup>c</sup>	2/26	4/23	6/18	2/18 <sup>b1</sup>	1/25 <sup>b1</sup>
Clausthal-Zellerfeld	0/15 <sup>b1</sup>	2/13	1/14	0/15	0/14 <sup>c</sup>	4/10	1/10	0/8 <sup>b1</sup>
Espagit-Hallschlag <sup>a</sup>	3/11	1/13	0/14 <sup>c</sup>	3/11	5/9 <sup>b2</sup>	0/4	10/4 <sup>b2</sup>	14/0 <sup>b2</sup>
Company 1 <sup>f</sup>	4/5 <sup>b2</sup>	3/6	3/6	0/9	1/8	2/7	0/7 <sup>e</sup>	0/9 <sup>b1</sup>
Company 5 <sup>f</sup>	0/4	0/4	1/3	1/3	2/1 <sup>c</sup>	–	2/0 <sup>e</sup>	1/1
Road and construction worker	2/3	2/3	1/4	0/5	0/5	0/5 <sup>c</sup>	0/5	0/5 <sup>b1</sup>
Engine driver	2/8	1/9	2/8	0/10	1/9	0/9	1/9	1/7
Foreman	1/3	1/3	0/4	0/4	1/3	0/3	1/3	1/2
Weapon disposal	2/9	0/11 <sup>e</sup>	0/11	1/10	3/8	0/4	7/4 <sup>b2</sup>	11/0 <sup>b2</sup>
Total: non-exp	7/25	9/23 <sup>e</sup>	7/25 <sup>d</sup>	2/30	4/27	6/23	2/23	1/27
Total: exp	3/22	2/23	0/25	3/22	5/19	4/9	11/9 <sup>b2</sup>	14/6 <sup>d</sup>

<sup>a</sup>The workers involved in the project Espagit-Hallschlag were all from company 6.

<sup>b1</sup>Prevalent no-cases in comparison to the exposed workers of all other companies.

<sup>b2</sup>Prevalent yes-cases in comparison to the exposed workers of all other companies.

<sup>c</sup>Prevalence of yes-cases comparing non-exposed and exposed workers of the same project.

<sup>d</sup>Significant difference between all non-exposed and exposed workers.

<sup>e</sup>Prevalent yes-cases in comparison to the exposed workers of all other companies ( $P < 0.05$ , calculated from  $\chi^2$  test, likelihood ratio).

<sup>f</sup>All workers of company 1 and 5 were involved in the project Stadtallendorf.

GGT, gamma-glutamyl-transpeptidase; MCV, mean corpuscular volume; SGPT, serum glutamic pyruvic transaminase.

For a comparison with threshold values published by regulatory authorities, the daily dose of the present workers was roughly estimated from the Hb-adduct levels. We assumed that the same percentage of the dose binds in human as in rats and that the steady-state level of the Hb adduct is equal to 60 times ( $0.5 \times$  lifetime of the human erythrocytes) the daily adduct level (Ehrenberg et al. 1974, Tannenbaum et al. 1986). For 4-aminobiphenyl, the percentage of the dose bound to Hb in rats was very similar to the percentage of the dose bound to Hb of smokers (Tannenbaum et al. 1986), although two additional cysteine groups are present in the alpha-chain of the Hb of rats (Maples et al. 1990) but not in humans or mice. The Hb-adduct levels for five arylamines were 2–28 times lower in mice than in rats (Birner & Neumann 1988). Therefore, the estimation of the daily dose from Hb adducts in rats could be an underestimation. In rats  $\sim 0.09\%$  of the dose binds to Hb (Zwirner-Baier et al. 1994). The daily dose for the median Hb-adduct level was estimated to be  $\sim 0.05 \mu\text{g/kg/day}$ . This is  $\sim 10$  times below the oral reference dose ( $0.5 \mu\text{g/kg daily}$ ) estimated by the US Environmental Protection Agency (IRIS 1991). Guideline values for drinking water have been set at  $0.2 \mu\text{g/l}$  by German authorities (Wollin & Dieter 2005). Assuming a 2 l per day consumption of water this would yield a daily dose of  $\sim 0.06 \mu\text{g/kg}$ . Therefore, the median Hb-adduct levels found in the adduct-positive workers of the present work appear to be safe. However, since more cases of positive samples were found with the urine analyses it would be more appropriate to generate a biological tolerance value for TNT metabolites in urine.

## Conclusions

According to the results of the present study, it appears that the blood parameter changes and the health effects are more influenced by other factors than by the internal exposure to TNT (Table VI).

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